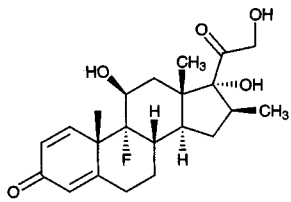
Betamethasone



Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.47

CAS Registry No.: 378-44-9, 987-24-6 (acetate), 22298-29-9 (benzoate), 5593-20-4 (dipropionate), 151-73-5 (sodium phosphate), 2152-44-5 (17-valerate), 5534-05-4 (acibutate), 360-63-4 (dihydrogen phosphate)

Merck Index: 1226

Lednicer No.: 1 198

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.277

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Condition a 500 mg diol SPE cartridge (Analytichem) with 6 mL dichloromethane. Sonicate a sample of cream containing 366 µg betamethasone valerate with 5 mL hexane:dichloromethane 70:30 for 5 min, make up to 10 mL with hexane:dichloromethane 70:30, add a 5 mL aliquot to the SPE cartridge, wash with 1 mL hexane:dichloromethane 70:30, elute with two 1 mL portions of MeOH, add 500 µL 180 µg/mL hydroxyresorcinol to the eluate, inject a 5 µL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: MeOH:water 75:25
Flow rate: 0.5
Injection volume: 5
Detector: UV 235

CHROMATOGRAM
Retention time: 10 (betamethasone valerate)
Internal standard: hydroxyresorcinol

OTHER SUBSTANCES
Simultaneous: chlorocresol

KEY WORDS
cream; SPE

REFERENCE
Di Pietra,A.M.; Andrisano,V.; Gotti,R.; Cavrini,V. On-line post-column photochemical derivatization in liquid chromatographic-diode-array detection analysis of binary drug mixtures, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1191–1199.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.2
Detector: UV 242

CHROMATOGRAM
Retention time: 5 (betamethasone 17-valerate)

REFERENCE
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

SAMPLE
Matrix: solutions
Sample preparation: Pass 20 mL of a solution in water (?) through an Empore C18 SPE disc. Wash with 2.5 mL water, dry, add 1 mL MeOH, let soak for 3 min, elute. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 1 mL running buffer, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5 ODS2
Mobile phase: MeOH:water 75:25
Flow rate: 1
Detector: UV 254

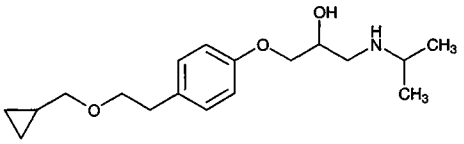
OTHER SUBSTANCES
Noninterfering: excipients

KEY WORDS
comparison with capillary electrophoresis; SPE

REFERENCE

Lucangioli,S.E.; Rodriguez,V.G.; Fernandez Otero,G.C.; Vizioli,N.M.; Carducci,C.N. Development and validation of capillary electrophoresis methods for pharmaceutical dissolution assays, *J.Capillary Electrophor.*, **1997**, *4*, 27–31.

Betaxolol



Molecular formula: $C_{18}H_{29}NO_3$
Molecular weight: 307.43
CAS Registry No.: 63659-18-7, 63659-19-8 (HCl)
Merck Index: 1229
Lednicer No.: 4 26

SAMPLE

Matrix: blood
Sample preparation: Add 5 mL chloroform:2-propanol:n-heptane 60:14:26, 1.5 mL saturated pH 9.5 ammonium chloride, and 40 μL 100 μg/mL prazepam to 2.0 mL postmorten blood. Shake horizontally for 20 min, centrifuge at 2800 g for 15 min. Evaporate organic phase under reduced pressure at 45°, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:tetrahydrofuran:100 mM pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 4.32
Internal standard: prazepan (8.81)
Limit of quantitation: 100 ng/mL

KEY WORDS

whole blood

REFERENCE

Berthault,F.; Kintz,P.; Tracqui,A.; Mangin,P. A fatal case of betaxolol poisoning, *J.Anal.Toxicol.*, **1997**, *21*, 228–231.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 300 μL MeOH:2-aminoheptane 99.7:0.3 at 1.5 mL/min, pass 700 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37

Flow rate: 1.2
Injection volume: 250
Detector: F ex 230 em 300

CHROMATOGRAM
Retention time: 15

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.4 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge at 13000 rpm for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 SynChropak bulk support (Knauer)
Column: 120 × 4.6 5 μm Spherisorb ODS1 C18
Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)
Flow rate: 1
Detector: UV 222

CHROMATOGRAM
Retention time: 5.02

OTHER SUBSTANCES
Extracted: cyclopropane carboxylic acid ester prodrug

KEY WORDS
plasma

REFERENCE
Hovgaard,L.; Brondsted,H.; Buur,A.; Bundgaard,H. Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents, *Pharm.Res.*, **1995**, *12*, 387–392.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 223

CHROMATOGRAM

Retention time: 7.38
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Blood or urine + 100 μL 200 ng/mL (blood) or 90 μL 1 μg/mL (urine) cicloprolol hydrochloride in water + 1 mL water + 200 μL 1 M NaOH, vortex, add 10 mL diethyl ether, shake on a mechanical shaker for 30 min, centrifuge at 1400 g for 15 min. Remove the organic layer and add it to 2 mL 50 mM HCl, mix for 10 min. Remove

the aqueous phase and add it to 200 μL 1 M NaOH, add 10 mL diethyl ether, mix for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-50°, reconstitute the residue in 100 μL 5 mM N,N-dimethyloctylamine, inject a 90 μL (blood) or 20-90 μL (urine) aliquot.

HPLC VARIABLES

Column: 75 × 4.5 3 μm Ultrasphere C18 (blood) or 150 × 4.5 5 μm Ultrasphere C18 (urine)
Mobile phase: MeCN:50 mM pH 3.0 N,N-dimethyloctylamine:water 8:10:82
Flow rate: 1
Injection volume: 20-90
Detector: F ex 200 no. 280 emission filter (Schoeffel Model FS 970)

CHROMATOGRAM

Retention time: 9 (blood), 29 (urine)
Internal standard: cicloprolol hydrochloride (6 (blood), 20 (urine))
Limit of detection: 10 ng/mL (urine), 1 ng/mL (blood)

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Wong,Y.W.J.; Ludden,T.M. Determination of βxolol and its metabolites in blood and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1990**, *534*, 161–172.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.405

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.3

OTHER SUBSTANCES
Also analyzed: alprenolol, bopindolol, propranolol, tertatolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.52, 1.57 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 9.86

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in 0.5% orthophosphoric acid.

HPLC VARIABLES

Column: 5 mm i.d. 4 μm Nova-Pak phenyl radial-Pak
Mobile phase: MeCN:MeOH:0.05% orthophosphoric acid 24:10:66
Flow rate: 1.6
Detector: F ex 261 em 306

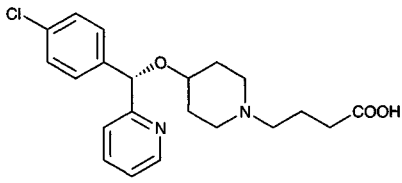
OTHER SUBSTANCES

Simultaneous: dextromethorphan, levorphanol (dextrorphan)

REFERENCE

Laslett,T.J.; Alvarez,F.; Nation,R.L.; Evans,A.M.; Scott,S.D.; Stupans,I. Effect of cyclophosphamide administration on the activity and relative content of hepatic P4502D1 in rat, *Xenobiotica*, **1995**, *25*, 1031–1039.

Betotastine



Molecular formula: $C_{21}H_{25}ClN_2O_3$
Molecular weight: 388.89
CAS Registry No.: 125602-71-3

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9.0 phosphate buffer and 100 μL water, add 8 mL chloroform and extract. (Caution! Chloroform is a carcinogen!) Evaporate the organic layer, dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2-fold the weight of water. Mix 1500 μL brain homogenate with 8 mL MeCN, centrifuge, evaporate 8 mL supernatant, dissolve the residue in 1 mL MeCN:water 50:50 and clean it up by a 1 mL 100 Bond Elute C18 SPE cartridge. Evaporate the effluent to dryness, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES

Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 17:83 (plasma), MeCN:0.018% TFA 18:82 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM

Limit of quantitation: 10 ng/mL (plasma), 35 ng/mL (brain)

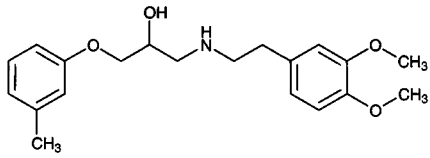
KEY WORDS

brain; cat; mouse; pharmacokinetics; plasma; rat; SPE

REFERENCE

Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

Bevantolol



Molecular formula: $C_{20}H_{27}NO_4$
Molecular weight: 345.44
CAS Registry No.: 59170-23-9, 42864-78-8 (HCl)
Merck Index: 1238
Lednicer No.: 3 28

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond-Elut C18 SPE cartridge with 1 volume MeCN and 1 volume 25 mM ammonium hydroxide, do not allow to dry. 500 µL Plasma + 80 µL water + 1 mL MeCN, vortex for 10 s, centrifuge at 2000 rpm for 10 min, remove the supernatant and add it to 1 mL 25 mM pH 10.5 ammonium hydroxide, vortex, add 100 µL 250 µg/mL 2,3,4,5-tetra-O-acetyl-α-D-glucopyranosyl isothiocyanate in MeCN (prepare fresh daily) with vortexing, let stand at room temperature for 10 min, add to SPE cartridge, wash with 1 volume 25 mM ammonium hydroxide, wash with 1 volume water, elute with 1.25 mL MeOH. Evaporate the eluent to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 µL mobile phase, inject a 75 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 µm Partisil 5 RAC II
Mobile phase: MeCN:75 mM $(NH_4)_2HPO_4$ adjusted to pH 3.5 with phosphoric acid 50:50
Column temperature: 45
Flow rate: 2
Injection volume: 75
Detector: UV 220

CHROMATOGRAM

Retention time: 6 (+), 7 (-)
Limit of detection: 20 ng/mL
Limit of quantitation: 40 ng/mL

KEY WORDS

plasma; chiral; SPE; derivatization

REFERENCE

Rose,S.E.; Randinitis,E.J. A high-performance liquid chromatographic assay for the enantiomers of bevantolol in human plasma, *Pharm.Res.*, **1991**, *8*, 758–762.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 9.5 sodium bicarbonate buffer + 100 ng pronethalol, extract with 5 mL diethyl ether:dichloromethane 60:40. Remove the organic layer and extract it with 200 µL 100 mM HCl, inject the aqueous phase. Urine. Incubate urine with pH 5.5 acetate buffer and β-glucuronidase at 35°, buffer with 1 M pH 9.5 sodium carbonate, extract with diethyl ether:dichloromethane 60:40. Remove the organic layer and extract it with 100 mM HCl, inject the aqueous phase.

HPLC VARIABLES

Column: 5 µm Spherisorb ODS-2
Mobile phase: MeCN:MeOH:500 mM pH 3.5 phosphate buffer 30:20:50
Injection volume: 200
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 3.0
Internal standard: pronethalol (plasma), verapamil (urine)
Limit of detection: 1000 ng/mL (urine), 50 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Nattel,S.; Lawand,S.; Matthews,C.; McCans,J. Bevantolol disposition in patients with hepatic cirrhosis, *J.Clin.Pharmacol.*, **1987**, *27*, 962–966.

SAMPLE
Matrix: feed
Sample preparation: 2 g Feed + 20 mL MeOH, rotate at 20 rpm for 1 h, centrifuge at 1300 rpm for 15 min, inject an aliquot.

HPLC VARIABLES
Guard column: 100 × 6.3 30-38 μm Co:Pell ODS (Whatman)
Column: 250 × 4.6 10 μm Lichrosorb RP-18
Mobile phase: MeOH:water:acetic acid 60:40:1 containing 5 mM sodium octanesulfonate (flush column with MeCN:water 50:50 after use)
Flow rate: 2
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 10
Limit of detection: 50 μg/g

KEY WORDS
complexation

REFERENCE
Spurlock,C.H.; Schneider,H.G. Liquid chromatographic and ultraviolet spectrophotometric determination of bevantolol and hydrochlorothiazide in feeds, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 321–324.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

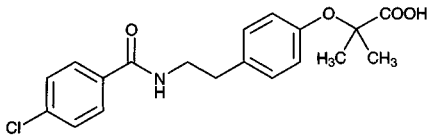
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 10:90:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.97, 4.10 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

Bezafibrate



Molecular formula: $C_{19}H_{20}ClNO_4$
Molecular weight: 361.82
CAS Registry No.: 41859-67-0
Merck Index: 1240
Lednicer No.: 3 44

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.268

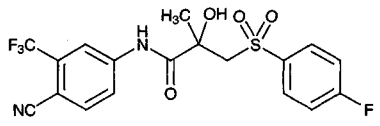
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bicalutamide



Molecular formula: $C_{18}H_{14}F_4N_2O_4S$
Molecular weight: 430.38
CAS Registry No.: 90357-06-5
Merck Index: 1247

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma, 1 mL 50 mM pH 7.0 phosphate buffer, 6 mL ethyl acetate, and 50 μL IS in MeOH, centrifuge. Remove a 5 mL portion of the organic layer and evaporate it to dryness. Reconstitute the residue in 400 μL MeCN:water 30:70, inject a 200 μL aliquot onto column A, elute with mobile phase A. Collect effluent containing the undifferentiated enantiomers, evaporate to dryness, reconstitute the residue in 400 μL MeCN:20 mM pH 7.0 phosphate buffer15:80. Inject a 200 μL aliquot onto column B. Elute with mobile phase B.

HPLC VARIABLES

Column: A 100 × 4.6 5 μm Hypersil ODS; B 5 μm AGP + 150 × 4.6 Ultron ES-OVM
Mobile phase: A MeCN:water 30:70; B MeCN:20 mM pH 7.0 phosphate buffer15:80
Injection volume: 200
Detector: UV

CHROMATOGRAM

Limit of detection: 5 ng/mL (R), 3.8 ng/mL (S)

KEY WORDS

plasma; pharmacokinetics; chiral

REFERENCE

Cockshott,I.D.; Oliver,S.D.; Young,J.J.; Cooper,K.J.; Jones,D.C. The effect of food on the pharmacokinetics of the bicalutamide ('casodex') enantiomers, *Biopharm.Drug Dispos.*, **1997**, *18*, 499–507.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize tissue in water. Buffer 500 μL intestinal homogenate with 1.5 mL 50 mM pH 7 phosphate buffer, add 6 mL ethyl acetate, mix, centrifuge. Remove a 5 mL portion of the organic layer and evaporate it to dryness, reconstitute the residue with 500 μL MeCN:water 50:50. Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 30:70
Injection volume: 50
Detector: UV

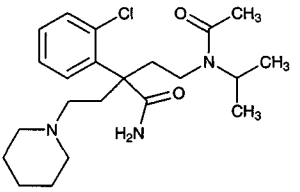
KEY WORDS

rat; jejunum; ileum; colon

REFERENCE

Cockshott,I.D.; Oliver,S.D.; Young,J.J.; Cooper,K.J.; Jones,D.C. The effect of food on the pharmacokinetics of the bicalutamide ('casodex') enantiomers, *Biopharm.Drug Dispos.*, **1997**, *18*, 499–507.

Bidisomide



Molecular formula: $C_{22}H_{34}ClN_3O_2$
Molecular weight: 407.98
CAS Registry No.: 103810-45-3
Merck Index: 1251

SAMPLE

Matrix: blood
Sample preparation: Alkalinize plasma with 1 M sodium hydroxide and extract with 1 mL chloroform (Caution! Chloroform is a carcinogen !). Centrifuge, transfer organic layer into tube and evaporate to dryness under a stream of dry nitrogen. Reconstitute samples with 200 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: Brownlee 5 µm CN
Mobile phase: MeCN:20 mM pH 4.0 monosodium phosphate 50:50
Flow rate: 1.0
Injection volume: 50
Detector: UV 207

CHROMATOGRAM

Internal standard: disopyramide
Limit of detection: 50 ng/mL

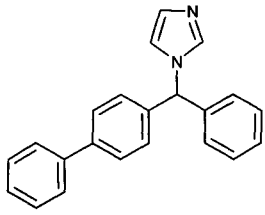
KEY WORDS

plasma; human; dog; pharmacokinetics

REFERENCE

Pao,L.-H.; Zhou,S.Y.; Cook,C.; Kararli,T.; Kirchhoff,C.; Truelove,J.; Karim,A.; Fleisher,D. Reduced systemic availability of an antiarrhythmic drug, bidisomide, with meal co-administration: Relationship with region-dependent intestinal absorption, *Pharm.Res.*, **1998**, *15*, 221–227.

Bifonazole



Molecular formula: $C_{22}H_{18}N_2$
Molecular weight: 310.40
CAS Registry No.: 60628-96-8
Merck Index: 1260
Lednicer No.: 4 93

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 1 mL MeOH, elute with 3 mL mobile phase. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 11
Internal standard: ketoconazole (7)

OTHER SUBSTANCES

Simultaneous: clotrimazole, ketoconazole, fenticonazole, tioconazole, isoconazole, econazole, miconazole

KEY WORDS

tablets; creams

REFERENCE

Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 85:15
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.6

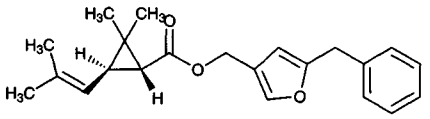
KEY WORDS

narrow-bore; chiral; α 1.56

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

Bioresmethrin



Molecular formula: $C_{22}H_{26}O_3$
Molecular weight: 338.45
CAS Registry No.: 28434-01-7
Merck Index: 1271

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:dichloromethane:isopropanol 99:1:0.07
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.56 (cis), k' 6.95 (trans)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, cyfluthrin (baythroid), permethrin, phenothrin, RU-11679, tetramethrin

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

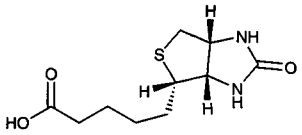
HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.95:0.05
Flow rate: 0.8
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: phenothrin, permethrin

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

Biotin



Molecular formula: $C_{10}H_{16}N_2O_3S$
Molecular weight: 244.31
CAS Registry No.: 58-85-5
Merck Index: 1272

SAMPLE

Matrix: blood, formulations

Sample preparation: Serum. Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 5 mL 1% acetic acid in water. 1 mL Serum + 1 mL 10% trichloroacetic acid, mix, centrifuge at 2000 g for 5 min, add a 1.5 mL aliquot of the supernatant to the SPE cartridge, wash with 10 mL 1% acetic acid in water, wash with 1 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness, reconstitute with 100 µL MeOH, sonicate, add 100 µL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, heat at 40° for 1 h, cool to room temperature, add 300 µL MeOH, inject a 10 µL aliquot. Tablets. Powder tablets, weigh out amount containing 500 µg biotin, add 30 mL MeOH, sonicate for 10 min, shake for 10 min, centrifuge at 3000 rpm for 5 min, remove the supernatant, repeat the extraction three times. Combine the supernatants and make up to 100 mL with MeOH. Remove a 200 µL aliquot and add it to 200 µL 5 mg/mL 1-pyrenyldiazomethane (Molecular Probes, Eugene OR) in ethyl acetate, heat at 40° for 1 h, cool to room temperature, add to a Sep-Pak silica SPE cartridge, wash with 5 mL hexane, elute with 10 mL MeOH. Evaporate the eluate to dryness, reconstitute with 1 mL MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 µm LiChrosorb Si60
Mobile phase: MeCN:water 43:57 (or 57:43 (?))
Flow rate: 1
Injection volume: 5-10
Detector: F ex 340 em 395, UV 240

CHROMATOGRAM

Retention time: 23
Limit of detection: 100 fmole

KEY WORDS

derivatization; tablets; SPE; serum

REFERENCE

Yoshida,T.; Uetake,A.; Nakai,C.; Nimura,N.; Kinoshita,T. Liquid chromatographic determination of biotin using 1-pyrenyldiazomethane as a pre-column fluorescent labelling reagent, *J.Chromatogr.*, **1988**, *456*, 421–426.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 8.89

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: feed, formulations
Sample preparation: Formulations. Dilute 50 μL liquid vitamin to 2 mL with mobile phase, filter, inject a 20 μL aliquot. Feed. Extract 100 mg horse feed with 10 mL 1 M NaOH. Remove a 6 mL aliquot and adjust the pH to 6-7 with 1 M HCl, dilute to 100 mL with 100 mM pH 6.0 phosphate buffer, filter, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C18
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: A was 100 mM pH 6.0 phosphate buffer. B was MeOH:200 mM pH 6.0 phosphate buffer 50:50. A:B was 54:46.
Flow rate: 0.4
Injection volume: 20
Detector: F ex 490 em 520 (photon-counting) following post-column reaction. Column effluent mixed with a 2 μg/mL solution of avidin-FITC (Sigma) in 100 mM pH 7.0 phosphate buffer pumped at 1 mL/min. The mixture flowed through a 10 m long by 0.5 mm i.d. length of knitted open tubular PTFE tubing to the detector.

CHROMATOGRAM

Retention time: 15
Limit of detection: 4.45 ng/mL

OTHER SUBSTANCES

Extracted: biocytin
Noninterfering: DMF, acetone, methyl ethyl ketone, vitamin A, ascorbic acid, vitamin D, vitamin E, pyridoxine, vitamin B12, thiamine, riboflavin, niacin, pantothenic acid

KEY WORDS

liquid vitamin; horse feed; complexation; post-column reaction

REFERENCE

Przyjazny,A.; Hentz,N.G.; Bachas,L.G. Sensitive and selective liquid chromatographic postcolumn reaction detection system for biotin and biocytin using a homogeneous fluorophore-linked assay, *J.Chromatogr.A*, **1993**, *654*, 79–86.

SAMPLE

Matrix: formula

Sample preparation: 20 mL Infant formula + 150 μL concentrated HCl, filter (Whatman No. 1 paper), rinse precipitate with 1 mL water, adjust the pH of the filtrate to 7.0 with 6 M NaOH, extract four times with 8 mL n-hexane, dilute the aqueous layer to 25 mL with water, inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C18

Column: 250 × 4.6 5 μm Microsorb C18

Mobile phase: MeOH:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 0.4

Injection volume: 20

Detector: F ex 495 em 518 following post-column derivatization. The effluent from the column mixed with reagent pumped at 0.1 mL/min and flowed through a 10 m × 0.5 mm i.d. PTFE tube in a knitted open-tubular reactor. The reagent was 2 μg/mL streptavidin-FITC (streptavidin labeled with fluorescein isothiocyanate 1:3.6, Vector Laboratories) in 100 mM pH 9.5 phosphate buffer, prepared fresh daily.)

CHROMATOGRAM

Retention time: 14

Limit of detection: 97 pg

OTHER SUBSTANCES

Extracted: biocytin

Noninterfering: niacinamide

KEY WORDS

post-column reaction; complexation

REFERENCE

Hentz,N.G.; Bachas,L.G. Class-selective detection system for liquid chromatography based on the streptavidin-biotin interaction, *Anal.Chem.*, **1995**, *67*, 1014–1018.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets or capsules, weigh out amount corresponding to 200 μg biotin, add 30 mL water, sonicate for10 min, make up to 50 mL with water, centrifuge at 2000 g for 10 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Tomsorb C18 (Tomsic, Tokyo, Japan)

Mobile phase: MeCN:buffer 5:95 (Buffer was 20 mM sodium dodecyl sulfate adjusted to pH 3.5 with perchloric acid.)

Column temperature: 40

Flow rate: 0.5

Injection volume: 20

Detector: F ex 342 em 542 following post-column reaction. The column effluent mixed with 0.03% sodium hypochlorite in 200 mM pH 12.5 borate buffer pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm I.D. stainless steel tube at 50°. The effluent from this tube mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm I.D. stainless steel tube at 50° to the detector. (Prepare the reagent by dissolving 800 mg o-phthalaldehyde in 20 mL EtOH, adding 1.5 mL 3-mercaptopropionic acid and making up to 500 mL with 200 mM pH 10.5 borate buffer.)

CHROMATOGRAM

Retention time: 27

Limit of detection: 10 ng
Limit of quantitation: 20 ng

KEY WORDS
post-column reaction; tablets; capsules; granules

REFERENCE
Nojiri,S.; Kamata,K.; Nishijima,N. Fluorescence detection of biotin using post-column derivatization with OPA in high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1357–1362.

SAMPLE
Matrix: formulations
Sample preparation: Grind 20 tablets, weight out amount equivalent to 100 μg biotin, add 60 mL 1.5% phosphoric acid, sonicate for 20 min in a water bath at 50°, shake mechanically for 20 min, add 15 mL MeCN, dilute to 100 mL with 1.5% phosphoric acid, mix. Filter through a 0.45 μm nylon filter membrane, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm YMC Octylsilane C8 (YMC, Wilmington, NC)
Mobile phase: MeCN:water adjusted to pH 2.2 with phosphoric acid 5:95
Column temperature: 50
Flow rate: 2
Injection volume: 150
Detector: UV 200

CHROMATOGRAM
Retention time: 20.5
Limit of quantitation: 500 ng/mL

KEY WORDS
tablets

REFERENCE
Ekpe,A.E.; Hazen,C. Liquid chromatographic determination of biotin in multivitamin-multimineral tablets, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1311–1315.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve crushed tablet with sonication in 25 mL pH 3.5 potassium phosphate, centrifuge at 10000 rpm for 5 min, filter (0.45 μm) a 2 mL aliquot. Add filtrate to a C18 Sep-Pak SPE cartridge, wash with 2 mL water, wash with 3 mL phosphate buffer, wash with 2 mL MeCN:phosphate buffer 5:95, elute with MeCN:phosphate buffer 15:85, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Vydac HS C18
Mobile phase: MeCN:10 mM pH 3.5 KH_2PO_4 10:90
Detector: UV 230

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 75 μg/mL

KEY WORDS
tablets; SPE

REFERENCE
Hudson,T.S.; Subramanian,S.; Allen,R.J. Determination of pantothenic acid, biotin, and vitamin B12 in nutritional products, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 994–998.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: caffeine, citric acid, folic acid, niacin, niacinamide, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: solutions
Sample preparation: Evaporate biological samples, add 10 μmoles dibenzo-18-crown-6, add 100 μmoles 2,4'-dibromoacetophenone (p-bromophenacyl bromide), add 25 mg anhydrous potassium carbonate, add 1.6 mL MeCN, reflux for 1 h. For bulk quantities take 55 mg biotin + 5 mL EtOH + 3 mL water, neutralize (phenolphthalein) with 20 mM KOH in MeOH, remove the solvent. Suspend in 5 mL MeCN, add 60 mg potassium carbonate, add 24 mg dibenzo-18-crown-6, add 166 mg 2,4'-dibromoacetophenone, reflux for 1 h.

HPLC VARIABLES
Column: 330 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 60:40
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 17.4
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: dethiobiotin

KEY WORDS
derivatization

REFERENCE
Desbene,P.-L.; Coustal,S.; Frappier,F. Separation of biotin and its analogs by high-performance liquid chromatography: convenient labeling for ultraviolet or fluorimetric detection, *Anal.Biochem.*, **1983**, *128*, 359–362.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, pantothenic acid, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995**, p. 21.

SAMPLE
Matrix: tissue
Sample preparation: Condition two Sep-Pak C18 SPE cartridges with 10 mL MeOH and 10 mL water. 2-3 g Gut tissue or liver + 5 mL 5% trichloroacetic acid + 5 nmole dethiobiotin, homogenize, centrifuge at 10000 g for 15 min, re-extract pellet with 5 mL 5% trichloroacetic acid twice. Combine the supernatants and add them to a SPE cartridge, wash with 10 mL MeCN:water 2:98, elute with 10 mL MeCN:water 15:85, add the eluate to a 70 × 8 column of 200-400 mesh Dowex 1x8 formate, wash with 10 mL water, wash with 10 mL 100 mM potassium formate, elute with 30 mL 100 mM potassium formate. Add the eluate to a SPE cartridge, wash with 10 mL water, elute with 10 mL methyl formate. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL 2.5 mM panacyl bromide (p-(9-anthroyl)phenacyl bromide) and 0.5 mM dibenzo-18-crown-6 in acetone, add 20-30 mg potassium carbonate, heat at 57° for 3 h, inject an aliquot. (Panacyl bromide is available from Molecular Probes, Eugene OR.)

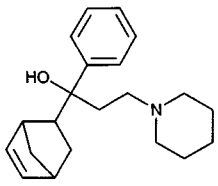
HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil
Mobile phase: Dichloromethane:MeOH 95:5
Flow rate: 1.4
Injection volume: 100
Detector: F ex 380 em 470

CHROMATOGRAM
Retention time: 6.46
Internal standard: dethiobiotin (5.56)

KEY WORDS
SPE; gut; rat; liver; normal phase

REFERENCE
Stein,J.; Hahn,A.; Lembcke,B.; Rehner,G. High-performance liquid chromatographic determination of biotin in biological materials after crown ether-catalyzed fluorescence derivatization with panacyl bromide, *Anal.Biochem.*, **1992**, *200*, 89–94.

Biperiden



Molecular formula: $C_{21}H_{30}ClNO$
Molecular weight: 311.47
CAS Registry No.: 514-65-8, 1235-82-1 (HCl), 7085-45-2 (lactate)
Merck Index: 1274
Lednicer No.: 1 47

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10.0 sodium carbonate buffer + 5 mL diethyl ether, shake vigorously for 5 min, centrifuge at 2000 rpm for 5 min. Remove the organic phase and add it to 2 mL 1 M HCl, shake for 2 min, centrifuge at 2000 rpm for 5 min. Remove the aqueous phase and add it to 1 mL 3 M NaOH, add 5 mL diethyl ether, shake for 2 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. (Extraction procedure from Chem. Pharm. Bull. 1985, 33, 4581.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Detector: UV 199

CHROMATOGRAM

Retention time: 5.6

OTHER SUBSTANCES

Simultaneous: hyoscyamine, orphenadrine, benztropine
Noninterfering: amantadine, carbidopa, levodopa
Interfering: bromocriptine

KEY WORDS

plasma

REFERENCE

Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.847

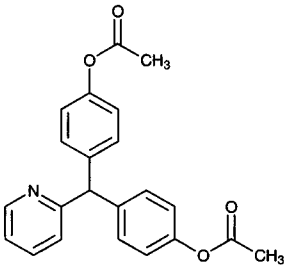
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bisacodyl



Molecular formula: $C_{22}H_{19}NO_4$
Molecular weight: 361.40
CAS Registry No.: 603-50-9, 1336-29-4 (complex with tannic acid)
Merck Index: 1282

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Grind tablets to powder, weigh out an amount equivalent to 5 mg bisacodyl, add 10-15 mL isopropanol, sonicate for 15 min, cool to room temperature, dilute to 25 mL with isopropanol, mix, centrifuge for 5 min. Remove a 5 mL aliquot and evaporate it to dryness under a stream of air, reconstitute the residue in 10 mL mobile phase, inject a 20 μL aliquot. Suppositories. Weigh out suppositories equivalent to about 50 mg bisacodyl, dissolve in warm (60°) isopropanol, cool to 15°, make up to 250 mL with isopropanol, mix, centrifuge an aliquot at 2000 rpm for 5 min. Remove 5 mL supernatant and evaporate it to dryness under a stream of air, dissolve in mobile phase, make up to 10 mL with mobile phase, centrifuge at 2000 rpm for 5 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:10 mM citric acid 25:25:50
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Limit of quantitation: 2500 ng/mL

KEY WORDS

tablets; suppositories

REFERENCE

Valenti,L.P.; Lau-Cam,C.A. Reverse phase liquid chromatographic determination of bisacodyl in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 529–532.

SAMPLE

Matrix: formulations
Sample preparation: Suppositories. Heat a suppository in 150 mL EtOH at 45° until it dissolves, cool to room temperature, make up to 200 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. Tablets. Shake 10 tablets in 10 mL buffer until tablets are soft, break up with a glass rod, add 170 mL EtOH, sonicate for 25 min with occasional shaking, cool to room temperature, make up to 200 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. Micro-enema. Sonicate micro-enema with 40 mL EtOH for 15 min, cool to room temperature, make up to 50 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. (Buffer was 2.88 g Na_2HPO_4 and 1.145 g KH_2PO_4 in 100 mL water.)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 60 RP-select B

Mobile phase: MeCN:50 mM KH_2PO_4 55:45
Flow rate: 1
Injection volume: 20
Detector: UV 214

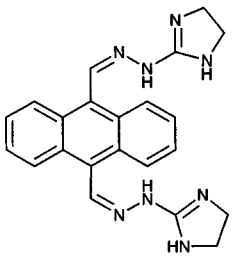
CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
suppositories; tablets; micro-enema

REFERENCE
Bradshaw,K.M.; Burnett,J.; Sidhu,A.S. High-performance liquid chromatographic determination of bisacodyl in pharmaceutical dosage forms marketed in Australia, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1355–1362.

Bisantrene



Molecular formula: $C_{22}H_{22}N_8$
Molecular weight: 398.47
CAS Registry No.: 78186-34-2, 71439-68-4 (di HCl)
Merck Index: 1284
Lednicer No.: 4 62

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Incubate 500 μL Bile or urine +50 μL 1 M pH 4.0 sodium acetate + 10 U/mL glucuronidase overnight. 2 mL Plasma or a lesser volume of urine or bile diluted to 1 mL with water + an equal volume of 1 M pH 10 sodium phosphate buffer + 1 μg IS + 8 mL ethyl acetate, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH with gentle warming, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 mm long 5 μm LiChrosorb RP-2 C2
Mobile phase: Gradient. MeOH:500 mM pH 5.3 sodium perchlorate from 5:95 to 100:0 over 15 min. (Purify sodium perchlorate solution by passing through a bed of silica gel and activated charcoal before use.)
Flow rate: 2
Injection volume: 100
Detector: UV 430 for bisantrene, UV 500 for IS

CHROMATOGRAM

Retention time: 7
Internal standard: 1-[2-(2-hydroxyethyl-1-amino)ethylamino]-4-hydroxy-9,10-anthracenedione
Limit of detection: 50 ng/mL (human plasma), 25 ng/mL (rabbit plasma)

KEY WORDS

plasma; rabbit; pharmacokinetics; human

REFERENCE

Powis,G. Reversed-phase high-performance liquid chromatographic assay for the antineoplastic agent 9,10-anthracenedicarboxaldehyde bis(4,5-dihydro-1H-imidazol-2-yl hydrazone) dihydrochloride, *J.Chromatogr.*, **1981**, *226*, 514–520.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 5% perchloric acid, mix, add 100 μL 25% ammonium hydroxide, add 4 mL ethyl acetate, vortex for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL 500 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: Reversed-phase C18 (Waters or Bio-Rad)
Mobile phase: MeOH:200 mM pH 4.0 ammonium acetate 40:60
Flow rate: 2
Detector: UV 260

CHROMATOGRAM

Retention time: 7

Limit of detection: 30 ng/mL

KEY WORDS
plasma

REFERENCE
Davis,T.P.; Peng,Y.-M.; Goodman,G.E.; Alberts,D.S. HPLC, MS, and pharmacokinetics of melphalan, bisantrene and 13-cis retinoic acid, *J.Chromatogr.Sci.*, **1982**, *20*, 511–516.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 10 mL MeOH and 5 mL water. Add 1-2 mL plasma to the SPE cartridge, wash with 5 mL water, elute with 300 μL 500 mM methanolic HCl, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS (Whatman)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:200 mM pH 4.0 ammonium acetate 27:73
Flow rate: 2
Detector: UV 436

CHROMATOGRAM
Retention time: 4
Limit of detection: 10 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Peng,Y.-M.; Ormberg,D.; Alberts,D.S.; Davis,T.P. Improved high-performance liquid chromatography of the new antineoplastic agents bisantrene and mitoxantrone, *J.Chromatogr.*, **1982**, *233*, 235–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL chloroform:MeOH:6 M HCl 83:16:1, agitate, centrifuge at 2000 g for 5 min, discard the organic phase, extract the aqueous phase with 3 mL chloroform and 100 μL 28% ammonium hydroxide, agitate, centrifuge. Remove 2.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, agitate, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:ammonium formate 30:60:5 (v/v/v)
Flow rate: 2
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 2.5
Internal standard: CL 238,985 (American Cyanamid analog of bisantrene) (4)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Weiss,G.R.; Hersh,M.; Kuhn,J.G.; Ludden,T.M.; von Hoff,D.D.; Kisner,D.L.; Pirtle,T.E.,III A phase I and pharmacokinetic comparison of hepatic arterial and peripheral vein infusions of bisantrene for liver cancer, *Cancer Chemother.Pharmacol.*, **1985**, *15*, 144–148.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 1 mL plasma to 11 with 50 μL 1 M NaOH, add 5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, centrifuge at 15600 g for 1 min, inject a 150 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 37-50 μm C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:80 mM pH 3.0 sodium formate 28:72
Flow rate: 1
Injection volume: 150
Detector: E, BAS LC-4B detector, TL-5 glassy carbon electrode at +0.75 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8
Internal standard: bisantrene

OTHER SUBSTANCES
Extracted: mitoxantrone

KEY WORDS
plasma; pharmacokinetics; bisantrene is IS

REFERENCE
Choi,K.E.; Sinkule,J.A.; Han,D.S.; McGrath,S.C.; Daly,K.M.; Larson,R.A. High-performance liquid chromatographic assay for mitoxantrone in plasma using electrochemical detection, *J.Chromatogr.*, **1987**, *420*, 81–88.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeOH, 4 mL MeOH:water 50:50, and 10 mL 50 mM 50 mM sodium phosphate. Add 3 mL plasma or urine to the SPE cartridge, wash with 4 mL 50 mM sodium phosphate, elute with 6 mL chloroform:MeOH 2:1. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL N,N-dimethylacetamide and 250 μL saline, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60 containing 20 mM ammonium acetate, pH 4.0
Flow rate: 1.5
Detector: F ex 260 em 550

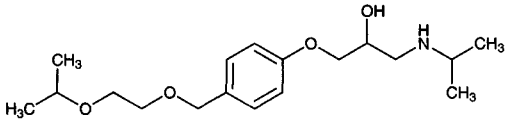
CHROMATOGRAM
Retention time: 8.5
Limit of detection: 2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE

Lu,K.; Savaraj,N.; Huang,M.T.; Moore,D.; Loo,T.L. High performance liquid chromatography (HPLC) of the new antineoplastic 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazole-2-yl)hydrazone] dihydrochloride (CL 216,942; bisantrene), *J.Liq.Chromatogr.*, **1982**, *5*, 1323–1328.

Bisoprolol



Molecular formula: $C_{18}H_{31}NO_4$
Molecular weight: 325.45
CAS Registry No.: 66722-44-9, 104344-23-2 (fumarate)
Merck Index: 1336
Lednicer No.: 4 28

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL pH 9.2 bicine (N,N-bis(2-hydroxyethyl)glycine) + 4 mL ethyl acetate, shake vigorously for 5 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, inject an aliquot of 100 μL or less.

HPLC VARIABLES

Guard column: 10 mm long LiChrosorb CN
Column: 250 × 4 10 μm LiChrosorb CN
Mobile phase: MeOH:isopropanol:1.16 M perchloric acid 75:25:0.5
Flow rate: 2.5
Injection volume: ≤100
Detector: F ex 235 em 310

CHROMATOGRAM

Retention time: 3.6
Internal standard: bisoprolol

OTHER SUBSTANCES

Extracted: sotalol
Simultaneous: verapamil
Noninterfering: acebutolol, amiodarone, disopyramide, propafenone, hydroquinidine, quinidine

KEY WORDS

plasma; bisoprolol is IS; pharmacokinetics

REFERENCE

Poirier,J.-M.; Lebot,M.; Cheymol,G. Rapid and sensitive column liquid chromatographic determination of sotalol in plasma, *J.Chromatogr.*, **1989**, *493*, 409–413.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30

Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 5.84
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Bond Elut SI SPE cartridge with 3 mL chloroform. 1 mL Plasma or 500 μL urine + 200 (plasma) or 500 (urine) μL water + 200 (plasma) or 300 (urine) μL 100 mM NaOH + 7 mL chloroform, shake for 10 min, centrifuge at 1800 g for 5 min. Remove the organic layer and add it to the SPE cartridge, wash with 1 mL MeOH, elute with 1 mL MeOH:triethylamine 95:5. Evaporate the eluate to dryness under

a stream of nitrogen at 60°, reconstitute the residue in 200 μL IS solution, inject a 100 μL aliquot. (Prepare IS solution by repeatedly injecting high concentrations of IS solution onto this HPLC system, collect the third peak, evaporate the collected eluates to dryness under a stream of nitrogen, reconstitute with mobile phase to an appropriate concentration.)

HPLC VARIABLES

Guard column: 50 × 4.6 10 μm Chiralcel OD (Daicel)
Column: 250 × 4.6 10 μm Chiralcel OD (Daicel)
Mobile phase: Hexane:isopropanol 100:9 containing 0.01% diethylamine
Column temperature: 25
Flow rate: 0.5
Injection volume: 100
Detector: F ex 228 em 298

CHROMATOGRAM

Retention time: 20 (R(+)), 30 (S(-))
Internal standard: 1-[p-(tetrahydro-3-furanyl)phenoxy]-3-isopropylamino-2-propanol (37)
Limit of detection: 2 ng/mL

KEY WORDS

plasma; chiral; SPE

REFERENCE

Suzuki,T.; Horikiri,Y.; Mizobe,M.; Noda,K. Sensitive determination of bisoprolol enantiomers in plasma and urine by high-performance liquid chromatography using fluorescence detection, and application to preliminary study in humans, *J.Chromatogr.*, **1993**, *619*, 267–273.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 12.283

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 50:50 (Buffer was 50 mM $(NH_4)_2HPO_4$ adjusted to pH 7.0 with 5 M orthophosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Retention time: 7
Limit of detection: 5-20 ng

OTHER SUBSTANCES
Simultaneous: fumaric acid, impurities

REFERENCE
Agapova,N.N.; Vasileva,E. High-performance liquid chromatographic method for the determination of bisoprolol and potential impurities, *J.Chromatogr.A*, **1993**, *654*, 299–302.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.85

OTHER SUBSTANCES
Also analyzed: carvedilol, labetalol, metipranolol, oxprenolol, talinolol, toliprolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD

Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.69, 1.38 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

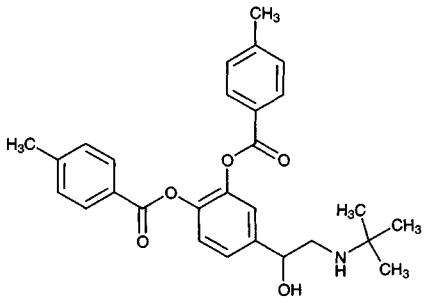
CHROMATOGRAM
Retention time: k' 4.43

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Bitolterol

Molecular formula: $C_{28}H_{31}NO_5$
Molecular weight: 461.56
CAS Registry No.: 30392-40-6, 30392-41-7 (mesylate)
Merck Index: 1344
Lednicer No.: 3 22



SAMPLE
Matrix: formulations
Sample preparation: Discharge aerosol into 10 mL MeOH in a 50 mL beaker until about 1.2 g sample is collected, rinse aerosol outlet with MeOH, determine weight of sample by difference in weight of can, dilute with MeCN:water:acetic acid 60:38:2 to about 20 μg/mL bitolterol, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:water:acetic acid:sodium octanesulfonate 600:380:20:0.65 (v/v/v/w)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: colterol, degradation products

KEY WORDS
aerosols

REFERENCE
Wilson,T.D.; Fogarty,D.F. The effect of column age on system suitability parameters for an HPLC assay of bitolterol mesylate aerosols, *J.Chromatogr.Sci.*, **1988**, *26*, 60–66.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:water:glacial acetic acid:sodium octanesulfonate 600:380:20:0.65
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (mesylate)

OTHER SUBSTANCES
Simultaneous: impurities, colterol

KEY WORDS
stability-indicating

REFERENCE

Wilson,T.D. High-performance liquid chromatographic determination of Tornalate in solution dosage forms; a specificity study, *J.Chromatogr.*, **1987**, *391*, 409–418.

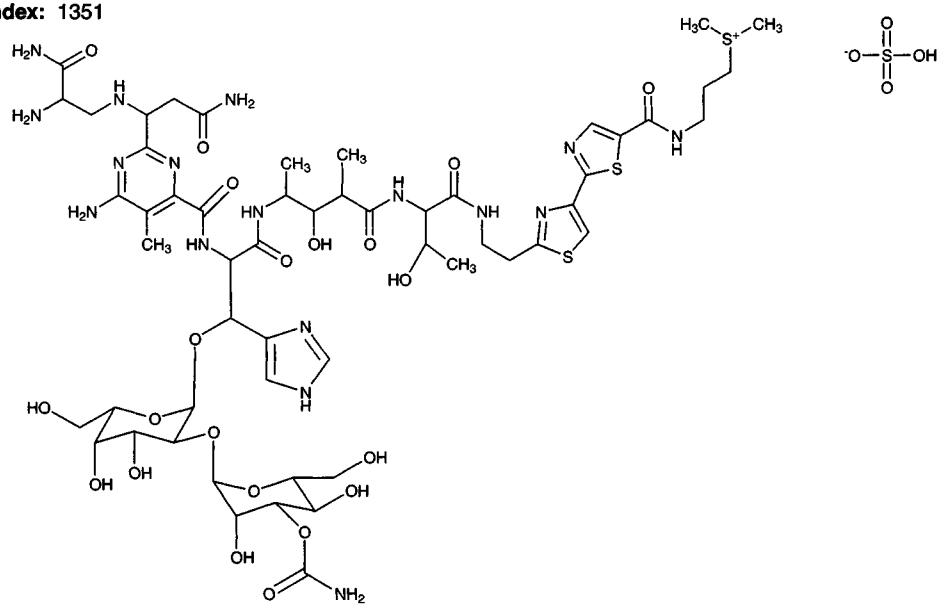
Bleomycin sulfate

Molecular formula: $C_{55}H_{84}N_{17}O_{21}S_3$ (bleomycin A_2)

Molecular weight: 1415.59

CAS Registry No.: 9041-93-4, 11056-06-7 (free base), 58995-26-9 (bleomycin A_1), 11116-31-7 (bleomycin A_2), 11116-32-8 (bleomycin A_5), 37293-17-7 (bleomycin A_6), 41138-54-9 (bleomycin B_1), 9060-10-0 (bleomycin B_2), 9060-11-1 (bleomycin B_4), 73666-80-5 (bleomycin B_6)

Merck Index: 1351



SAMPLE

Matrix: blood, hepatocytes

Sample preparation: 300 μL Plasma or hepatocyte suspension + 0.9 μg peplomycin + 75 μL trichloroacetic acid:water 20:80 containing 1 mM copper sulfate, vortex for 1 min, centrifuge at 850 g for 10 min, rewash the pellet with 75 μL trichloroacetic acid:water 20:80 containing 1 mM copper sulfate, vortex for 1 min, centrifuge at 850 g for 10 min. Combine the supernatants, make up to 300 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 37 × 3.9 Corasil C18

Column: 250 × 10 7 μm Lichrosorb RP-18

Mobile phase: Gradient. A was 5 mM sodium pentanesulfonate in 0.5% acetic acid, adjusted to pH 4.3 with 28% ammonia. B was MeOH:MeCN 25:75 containing 5 mM sodium pentanesulfonate and 0.5% acetic acid. A:B from 87:13 to 67:33 over 20 min

Column temperature: 35

Flow rate: 1.8

Injection volume: 100

Detector: F ex 297 em 355

CHROMATOGRAM

Retention time: 7 (deamidobleomycin A2), 8 (bleomycin A2), 9 (deamidobleomycin B2), 10 (bleomycin B2)

Internal standard: peplomycin (14.5)

Limit of detection: 70 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Mahdadi,R.; Kenani,A.; Pommery,N.; Pommery,J.; Hénichart,J.P.; Lhermitte,M. High-performance liquid chromatography assay of bleomycin in human plasma and rat hepatocytes in culture, *Cancer Chemother.Pharmacol.*, **1991**, *28*, 22–26.

SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 10000 ng/mL

REFERENCE
Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.6 μBondapak C18
Mobile phase: Gradient. All solvents contained 5 mM ammonium formate. MeOH:water 15:85 for 2 h, to 30:70 over 1 h, to 95:5 over 1.5 h.
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 80 (bleomycin A2), 150 (bleomycin B2), 200 (bleomycin A1), 240 demethylbleomycin A2)

REFERENCE
Rzeszotarski,W.J.; Eckelman,W.C.; Reba,R.C. Reversed-phase high-performance liquid chromatography of bleomycin, *J.Chromatogr.*, **1976**, *124*, 88–91.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeOH:water 50:50 containing 5 mM heptanesulfonic acid, adjust pH to 8.3 with concentrated ammonium hydroxide
Flow rate: 1.5
Detector: UV 290

CHROMATOGRAM
Retention time: 6 (bleomycin B2), 12 (bleomycin A2)
Limit of detection: 10 pmole

REFERENCE
Sakai,T.T. Paired-ion high-performance liquid chromatography of bleomycins, *J.Chromatogr.*, **1978**, *161*, 389–392.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in water, add a slight molar excess of copper sulfate, inject a 10-20 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 30-38 μm Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 5 mM sodium pentanesulfonate in 0.5% glacial acetic acid in water, adjusted to pH 4.3 with concentrated ammonium hydroxide. B was MeOH containing 5 mM sodium pentanesulfonate and 0.5% glacial acetic acid. A:B from 72:28 to 52:48 over 45 min.
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 280

CHROMATOGRAM
Retention time: 5 (bleomycinic acid), 10 (bleomycin B'1), 11.5 (bleomycin A2), 12.5 (bleomycin A5), 17.5 (bleomycin B2), 22.5 (bleomycin B4), 25.5 (bleomycin B6), 29 (bleomycin CHP), 32 (bleomycin PEPP), 35 (demethylbleomycin A2)
Limit of detection: 50 pmole

KEY WORDS
chelates

REFERENCE
Klett,R.P.; Chovan,J.P.; Danse,I.H.R. Reversed-phase paired-ion high-performance liquid chromatographic method for the separation and quantification of multiple bleomycin congeners, *J.Chromatogr.*, **1984**, *310*, 361–371.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in water, add a slight molar excess of copper sulfate, inject a 10-20 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 30-38 μm Corasil C18
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was 5 mM sodium heptanesulfonate in 0.5% glacial acetic acid in water, adjusted to pH 4.3 with concentrated ammonium hydroxide. B was MeOH containing 5 mM sodium heptanesulfonate and 0.5% glacial acetic acid. A:B from 40:60 to 50:50 over 30 min.
Flow rate: 0.7
Injection volume: 10-20
Detector: UV 280

CHROMATOGRAM
Retention time: 5 (epibleomycin A2), 8 (epibleomycin B2), 11 (desamidobleomycin A2), 12 (bleomycin A2), 14 (isobleomycin A2), 18 (desamidobleomycin B2), 20 (bleomycin B2), 21 (isobleomycin B2), 22 (epidemethylbleomycin A2), 24 (desamidodemethylbleomycin A2), 25.5 (demethylbleomycin A2), 28 (isodemethylbleomycin A2)

KEY WORDS
chelates

REFERENCE
Klett,R.P.; Chovan,J.P. Modification of a new high-performance liquid chromatographic method for bleomycin to separate epi-, iso-, desamido-, and unmodified analogs, *J.Chromatogr.*, **1985**, *337*, 182–186.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 50 × 5 10 μm Mono S HR 5/5 cation-exchange (Pharmacia LKB)
Mobile phase: Gradient. A was 50 mM pH 6.5 ammonium formate. B was 1 M pH 6.5 ammonium formate. A:B from 98:2 to 95:5 over 30 min, to 75:25 over 20 min, to 0:100 over 10 min.
Column temperature: 4
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 12 (bleomycin A2), 41 (bleomycin B2)

REFERENCE
Mistry,J.S.; Sebti,S.M.; Lazo,J.S. Separation of bleomycins and their deamido metabolites by high-performance cation-exchange chromatography, *J.Chromatogr.*, **1990**, *514*, 86–90.

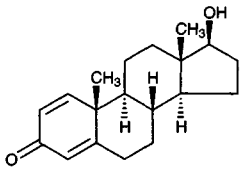
SAMPLE
Matrix: solutions
Sample preparation: Make up a 1 mg/mL (1 U/mL) aqueous solution, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher RP-Select B
Column: 125 × 4 5 μm LiChrospher RP-Select B
Mobile phase: Gradient. A was 10 mM sodium perchlorate in 0.1% aqueous phosphoric acid. B was MeCN. A:B from 95:5 to 75:25 over 13 min, to 0:100 over 1 min, maintain at 0:100 for 1 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5 (bleomycin A2), 7.8 (bleomycin B2), 12.3 (demethylbleomycin A2)
Limit of detection: 10 μg/mL

REFERENCE
Fiedler,H.-P.; Wachter,J. High-performance liquid chromatographic determination of bleomycins, *J.Chromatogr.*, **1991**, *536*, 343–347.

Boldenone



Molecular formula: $C_{19}H_{26}O_2$
Molecular weight: 286.41
CAS Registry No.: 846-48-0, 13103-34-9 (undecylenate)
Merck Index: 1354
Lednicer No.: 2 153

SAMPLE

Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone
Interfering: fluoxymesterone, norethindrone, oxandrolone (UV 210), ethisterone

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 4.5 (boldenone undecylenate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: testosterone undecenoate, nandrolone decanoate, methandriol dipropionate, testosterone decanoate, nandrolone laurate, testosterone undecanoate, testosterone, methenolone acetate, testosterone propionate, nandrolone phenylpropionate, testosterone phenylpropionate, testosterone isocaproate, trenbolone hexahydrobenzylcarbonate
Interfering: testosterone enanthate, methenolone enanthenate, testosterone cypionate

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 6.8 (boldenone), 13.4 (boldenone acetate), 25.1 (boldenone undecylenate)

OTHER SUBSTANCES
Simultaneous: clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 75:25
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5 (boldenone), 5.5 (boldenone acetate), 16 (boldenone benzoate)

OTHER SUBSTANCES
Also analyzed: nandrolone, nandrolone propionate, nandrolone phenylpropionate

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, testosterone, danazol, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.091

OTHER SUBSTANCES
Simultaneous: epimethandienone, epitestosterone, fluoxymesterone, 6β-hydroxymethandienone, methandienone, norethindrone, oxymetholone (UV 280), trenbolone

REFERENCE
Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg 40 μm C18 SPE cartridge (J.T.Baker model 7020-2) with 2 mL MeOH and 1 mL 25 mM pH 5.5 ammonium acetate (A). Condition a 200 mg 40 μm C18 SPE cartridge (J.T.Baker model 7020-2) with 2 mL MeOH and 1 mL water (B). 2 mL Urine + 2 mL MeOH:650 mM pH 5.4 ammonium acetate 20:80 + 75 μL 25.3 μM 19-nortestosterone sodium sulfate in water, sonicate for 15 min, centrifuge at 1000 g for 5 min, add to SPE cartridge (A), wash with 1 mL 25 mM pH 5.5 ammonium acetate, wash with 2 mL MeOH:25 mM pH 5.5 ammonium acetate 40:60, wash with 3 mL water, elute with 2 mL MeOH:water 35:65. Add 2 mL MeOH to the eluate and evaporate it under a stream of nitrogen at 60°, dissolve the residue in 100 μL MeOH, add 5 mL ethyl acetate saturated with 2.2 M sulfuric acid (one tenth volume), heat at 50° for 50 min, cool, wash twice with 2 mL 940 mM pH 10.3 sodium carbonate (pH adjusted with sodium bicarbonate), wash with 3 mL water. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, transfer the residue with three 200 μL portions of MeOH:water 50:50 to SPE cartridge (B), wash with 1 mL water, wash with 2 mL MeOH:water 55:45, elute with 2 mL MeOH:water 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Boldenone sulfate, isolated using SPE cartridge (A), is solvolysed to boldenone which is purified using SPE cartridge (B).)

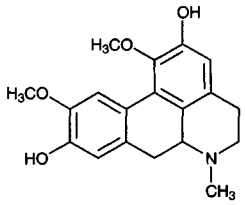
HPLC VARIABLES
Column: 83 × 4.6 3 μm Pecosphere-3C C18
Mobile phase: MeCN:MeOH:25 mM pH 5.5 ammonium acetate 7:50:43
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2
Internal standard: 19-nortestosterone sodium sulfate (7.2) (chromatographed as 19-nortestosterone)
Limit of detection: 64 ng/mL
Limit of quantitation: 212 ng/mL

KEY WORDS
horse; pharmacokinetics; SPE

REFERENCE
Weidolf,L.O.G.; Chichila,T.M.P.; Henion,J.D. Screening, confirmation and quantification of boldenone sulfate in equine urine after administration of boldenone undecylenate (Equipoise™), *J.Chromatogr.*, **1988**, *433*, 9–21.

Boldine



Molecular formula: $C_{19}H_{21}NO_4$
Molecular weight: 327.38
CAS Registry No.: 476-70-0
Merck Index: 1355

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM

Retention time: 8.068

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.